US Army Corps of Engineers
Waterways Experiment Station

Zebra Mussel Research Technical Notes

September 1998

Effects of Elevated Carbon Dioxide Concentrations on Survivorship in Zebra Mussels *(Dreissena polymorpha)*

Background and purpose

Many aquatic invertebrate animals, including bivalves, are intolerant of even relatively modest increases in the ambient concentration of carbon dioxide (CO_2). This intolerance is based on the chemical reaction of CO_2 with water to form carbonic acid. Carbon dioxide has the potential to be used as a molluscicide for zebra mussel control because these organisms, like almost all bivalves, do not contain the oxygen-carrying proteins to buffer blood pH. Instead, they less efficiently mobilize shell CO_3^{2-} as the main blood buffer.

Previous studies have indicated that zebra mussels could be sensitive to elevated carbon dioxide. Pretreatment with CO_2 greatly increased the mortality rate of mussels on subsequent exposure to lethal levels of chlorine. It was suggested that initial treatment with carbon dioxide induced valve gaping, which increased mussel sensitivity to chlorination. Carbon dioxide is relatively inexpensive, nonhazardous to humans, environmentally neutral, and readily and rapidly biodegraded by photosynthetic organisms.

Additional information

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The research described in this technical note was based on studies conducted by Drs. Robert F. McMahon, Milton A. Matthews, Lawrence R. Shaffer, and Paul D. Johnson, University of Texas at Arlington (McMahon and others 1995).

Approach

Tolerance to elevated carbon dioxide concentrations was determined for zebra mussels acclimated to 25 °C. Three replicate samples of zebra mussels (n = 25-30) were kept in water made anoxic by bubbling with 100 percent CO_2 or 100 percent N_2, or in water made normoxic by bubbling with air.

In a second experiment, three replicate samples (n = 25-30) were maintained in water made anaerobic by bubbling with a mixture of either 5 percent CO_2 and 95 percent N_2 (hypercapnic anoxia, P_{CO2} = 38 torr) or 100 percent N_2 (anoxia).

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A third experiment involved exposure of samples to elevated carbon dioxide concentrations in combination with approximately ambient air oxygen concentrations. Again, three replicate samples (n = 25-30) were kept in water bubbled with either a gas mixture of 5 percent CO_2:19 percent O_2:76 percent N_2 (hypercapnic, P_{CO2} = 38 torr; normoxic, P_{O2} = 144 torr) or with air (normocapnic, P_{CO2} = 2.5 torr; normoxic, P_{O2} = 150-160 torr).

In a fourth experiment, zebra mussels were exposed to hypercapnia of P_{CO2} = 76 torr under normoxic conditions. In this experiment, replicate samples of zebra mussels (n = 25) were exposed to water bubbled with either a hypercapnic, normoxic gas mixture of 10 percent CO_2:19 percent O_2:71 percent N_2 (P_{CO2} = 76, P_{O2} = 144 torr) or with air (normocapnic normoxia, P_{CO2} = 2.5 torr; normoxic, P_{O2} = 150-160 torr).

Viability of individuals in all experiments was recorded every 12 to 24 hr. The posterior mantle edges and siphons of all gaping zebra mussels were gently prodded with the tip of a blunted dissection needle. Individuals that failed to close their valves were considered to be dead. Viability testing continued until 100 percent mortality was achieved or until it was clear that test gas concentrations were not toxic.

When zebra mussels were exposed to hypercapnic, normoxic gas mixtures of 5 percent CO_2:19 percent O_2:76 percent N_2 , they did not exhibit mortality. However, they did have a tendency to become detached from the byssus and, once detached, were unable to reform a byssal attachment.

To further quantify this observation, mussels were removed from the byssal attachments by cutting their byssal threads at the byssal shell gape with razor blades. The mussels were placed on the upper surfaces of 14-cm by 14-cm by 2-mm clear plastic plates and allowed to byssally reattach over a 12-hr period. The new byssal threads were then counted by viewing through the underside of the plate at 30× with a dissecting microscope and were marked with a permanent ink marker. Plates were then placed in holding chambers similar to those used in the previous studies and acclimated to 25 °C. Water was bubbled with either a hypercapnic, normoxic gas mixture (5 percent CO_2:19 percent O_2: 76 percent N_2) or with air. Over an 11-day period, plates were removed daily, and the byssal mass of all attached mussels was examined as described above. New byssal attachments were counted and marked as above, allowing the daily rate of byssal thread production to be recorded for each individual. The number of individuals that detached from the byssal holdfast was also recorded.

Results

When exposed to anoxia in water bubbled with 100 percent CO_2, zebra mussels displayed significantly greater mortality rates than when exposed to anoxia induced by bubbling water with 100 percent N_2 (Figure 1). Mean mortality time of zebra mussels exposed to anoxia under 100 percent N_2 was 103.7 hr, and mortality when exposed to 100 percent CO_2 was 43.6 hr. Individuals did not exhibit a significant difference in time to death when exposed to either 100 percent N_2 or 5 percent CO_2:95 percent N_2 (Figure 2). Mean time to death for individuals exposed to 100 percent N_2 was 96.4 hr, and 91 hr for individuals exposed to 5 percent CO_2:95 percent N_2. Corresponding LT_{50} values were 83.2 hr under 5 percent CO_2:95 percent N_2, and 84.6 hr under 100 percent N_2. Corresponding SM_{100} values were 209 and 244 hr.

No significant mortality (<5 percent) was observed in three replicate samples of zebra mussels exposed to water made hypercapnic and normoxic, or only normoxic, by bubbling with either a gas mixture of 5 percent CO_2:19 percent O_2:76 percent N_2 (hypercapnic, P_{CO2} = 38 torr; normoxic, P_{O2} = 144 torr) or

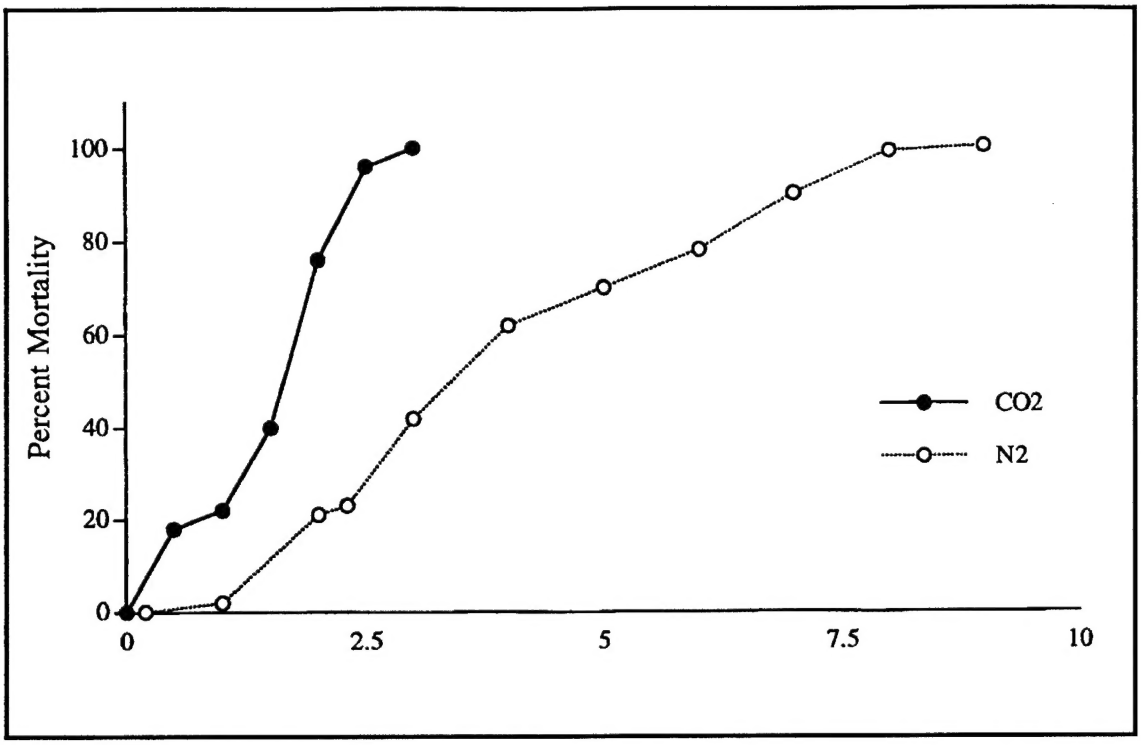

Figure 1. Zebra mussel mortality when kept in water at 25 °C made anoxic by continuously bubbling with either pure nitrogen or pure carbon dioxide

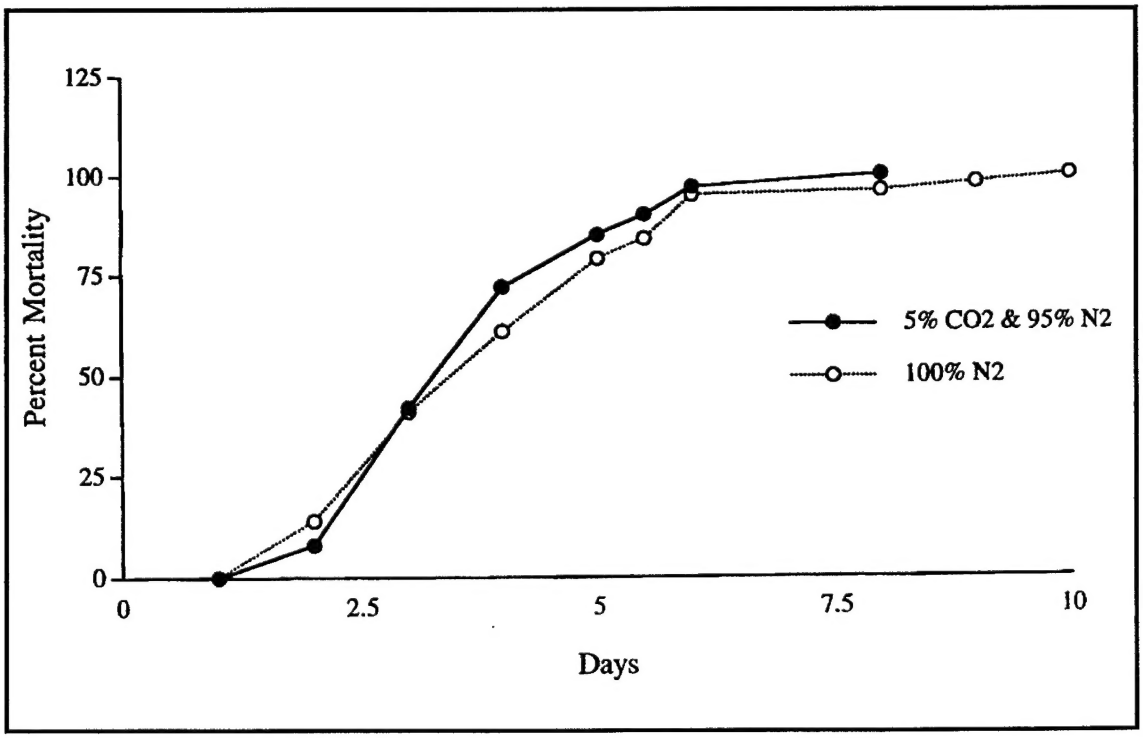

Figure 2. Zebra mussel mortality when kept in water at 25 °C made anoxic by continuously bubbling with either pure nitrogen or a hypercapnic mixture of 5 percent carbon dioxide and 95 percent nitrogen

with air at 25 °C over a 40-day period. While exposure to hypercapnic water with a P_{CO2} of 38 torr did not induce mortality, exposed individuals appeared stressed, gaping the valves widely and becoming detached from byssal threads.

In contrast to the lack of mortality on exposure to hypercapnic normoxia under a P_{CO2} of 36 torr, three replicate samples of adult mussels (n = 25) all experienced 100 percent mortality when exposed to water bubbled with a hypercapnic, normoxic gas mixture of 10 percent CO_2:19 percent O_2:71 percent N_2 over a 641-hr period at 25 °C.

Exposure of adult zebra mussels to water bubbled with a hypercapnic, normoxic gas mixture of 5 percent CO_2:19 percent O_2:76 percent N_2 (P_{CO2} = 38 torr) resulted in suppression of byssal thread production. During the 12-hr period over which mussels were allowed to byssally attach to plastic plates in water bubbled with air, both samples subjected to hypercapnic gas and air treatments produced equal numbers of byssal threads (hypercapnic water = 11.2 byssal threads; air-bubbled water = 11.8 byssal threads). After 24 hr in water bubbled with a hypercapnic gas mixture of 5 percent CO_2:19 percent O_2:76 percent N_2, byssal thread production in the hypercapnic medium was completely inhibited by the seventh day of exposure (Figure 3). In contrast, mussels in air-bubbled controls produced byssal threads daily throughout the exposure period, with production rate declining to 1.67 threads per day on the eleventh and final day of exposure (Figure 3).

Exposure to a 5 percent CO_2:19 percent O_2:76 percent N_2 mixture of gas in water also induced detachment from the byssal holdfast. In these cases, individuals did not form reattachments to the plastic plate or walls of the holding tank, and none remained attached at the end of 10 days (Figure 4). Individuals held in air-bubbled water also detached from the byssus, with only 31.6 percent remaining attached to the byssal holdfasts they occupied at the beginning of the exposure period. However, individuals in air-bubbled water moved to other locations and byssally reattached, a behavior commonly observed in laboratory amount healthy stocks of mussels.

Implications for control

More rapid deaths of zebra mussels were observed in water made anoxic by continuous bubbling with 100 percent CO_2 relative to 100 percent N_2. These results suggest that the mechanism of death was different for individuals bubbled in CO_2 compared with individuals bubbled in N_2. Under the anaerobic conditions induced by N_2 or CO_2 bubbling, zebra mussels depend on anaerobic metabolism to maintain vital metabolic functions. Anaerobic metabolism produces acidic end-products that could decrease body fluid pH to deleterious levels, although these materials are excreted to the surrounding water before they reach lethal concentrations. This allows bivalves to tolerate relatively long periods of anoxia.

In contrast, when bivalves are held in anoxic conditions under atmospheres of pure CO_2, diffusion of CO_2 into tissues probably results in decreased body fluid pH. This acidosis is compounded by release of acidic anaerobic end-products. Hemolymph acidosis would be further compounded by the decrease in external medium pH associated with high levels of dissolved CO_2 reacting with water to form H^+ and HCO_3^-. Resulting acidosis would reduce the ability of mussels to buffer blood pH, since they are dependent on mobilization of shell carbonate to buffer the hemolymph. Hemolymph pH reduction would result in a decrease in intracellular pH, inactivating essential metabolic enzymes that would lead to relatively rapid death. Rapid death recorded under 100 percent CO_2 was unlikely to be induced by the reduction in water pH alone since zebra mussels

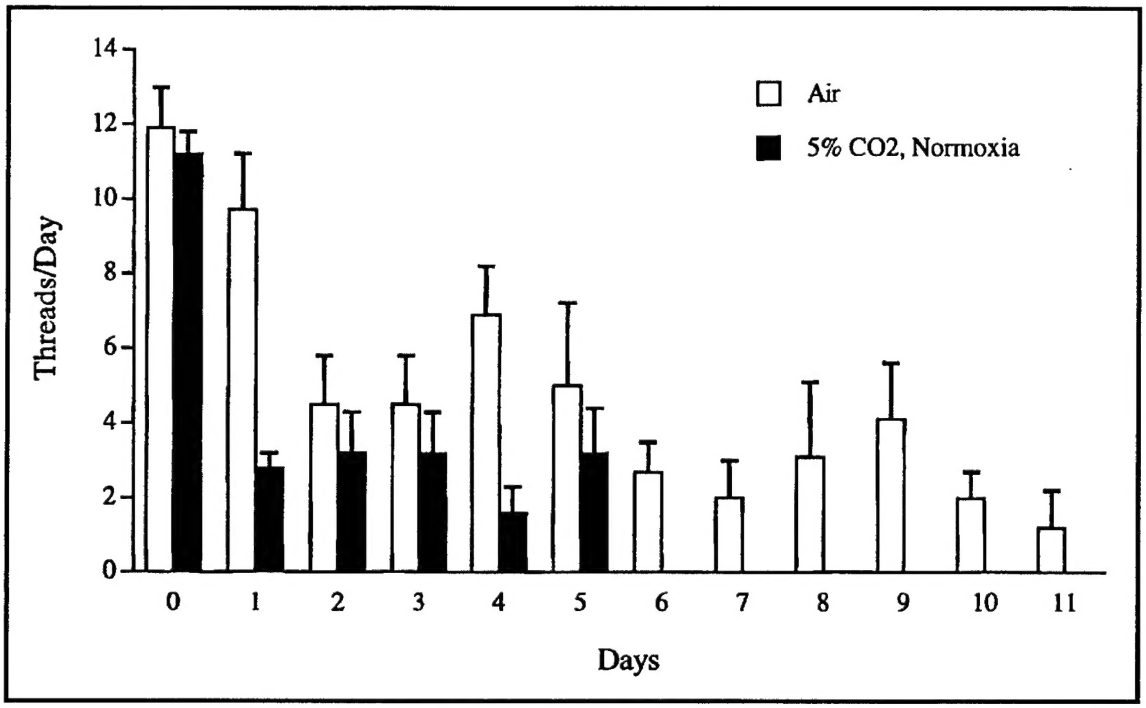

Figure 3. Mean number of byssal threads produced per day by adult zebra mussels kept in water at 25 °C made hypercapnic by continuously bubbling with a gas mixture of 5 percent CO_2:19 percent O_2: 76 percent N_2 (P_{CO2} = 38 torr, P_{O2} = 144 torr) (solid histograms) or in water continuously bubbled with air (open histograms). Vertical bars above histograms represent standard deviations of the means

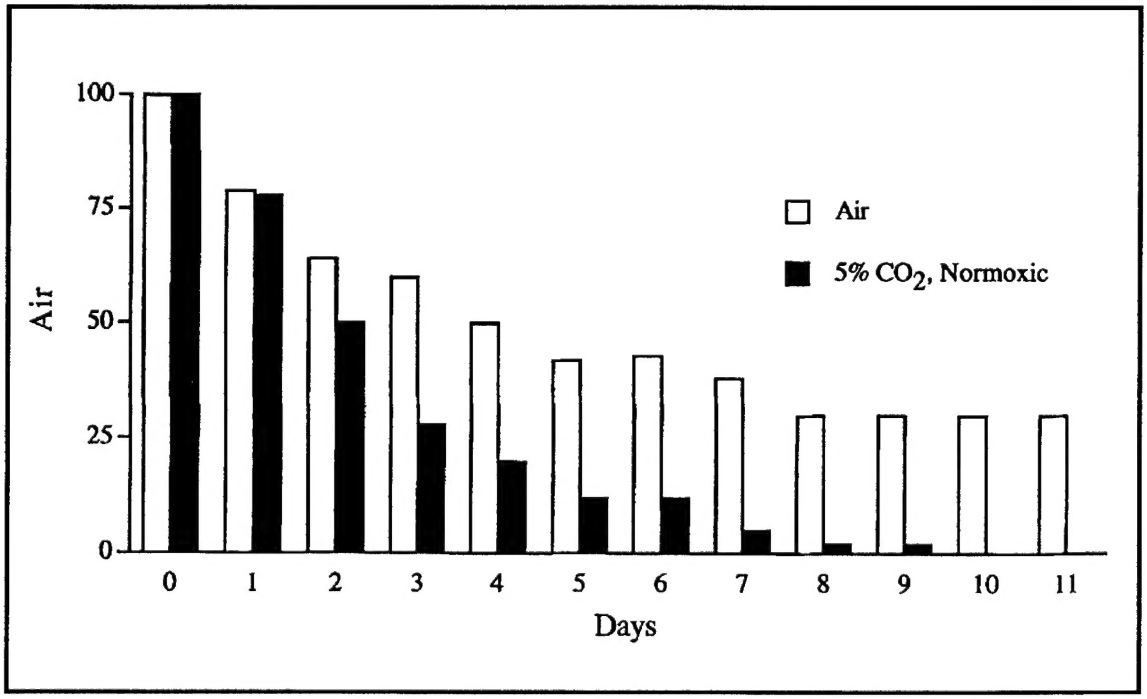

Figure 4. Percent of samples of adult zebra mussels remaining byssally attached during exposure to normoxic water at 25 °C made hypercapnic by continuously bubbling with a gas mixture of 5 percent CO_2: 19 percent O_2:76 percent N_2 (P_{CO2} = 38 torr, P_{O2} = 144 torr) (solid histograms) or to water continuously bubbled with air (open histograms)

have been found to tolerate this level of pH for far longer than those required to achieve 100 percent mortality under 100 percent CO_2.

This research indicates that CO_2 injection may have efficacy for control of zebra mussel raw water system macrofouling, both when applied periodically in high concentrations as a mitigating agent to off-line, isolated, or low-flow systems, or when applied continuously in low concentrations as a preventative agent to on-line high- and low-flow systems.

Carbon dioxide offers a number of advantages as a molluscicide. It is relatively inexpensive and readily available from commercial suppliers. It can be easily transported and stored onsite and is nonhazardous to humans whether leaked to the atmosphere or in solution. Carbon dioxide is a natural product that can be easily removed through fixation into organic compounds by photosynthesis.

The primary disadvantage of carbon dioxide is that relatively large quantities are required to induce effective mitigation compared with molluscicides currently used. Also, since CO_2 is a weak acid, it could increase metallic corrosion rates. However, it could be used by companies that generate large quantities of waste CO_2. Used as a preventative measure, continuous application of carbon dioxide at levels of P_{CO2} = 38 torr or 76 torr probably will not lower the pH of naturally alkalotic water inhabited by zebra mussels below neutrality, thus minimizing the corrosion rates of raw water system metallic components.

Results of these experiments indicate that carbon dioxide can cause mussel mortality and limit byssal thread production. It offers the potential for zebra mussel control at water-based facilities that choose to pursue this approach.

Reference

McMahon, R. F., Matthews, M. A., Shaffer, L. R., and Johnson, P. D. (1995). "Effects of elevated carbon dioxide concentrations on survivorship in zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*)." *Fifth international zebra mussel and other aquatic nuisance organisms conference.* 319-36.